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Determination of other titratable alkaloids in the morphine of opium assays

In continuation of the international programme of scientific research undertaken pursuant to resolutions 159 II C (VII) and 246 F (IX), adopted by the Economic and Social Council on 3 August 1948 and 6 July 1949 respectively, the Secretary-General is presenting the following paper prepared by the United Nations Secretariat chemists. The assay of opium is subject to both positive and negative errors, which vary with the method of assay. The present paper deals particularly with the determination of the positive error due to the presence of other titratable alkaloids contaminating the assay morphine in both precipitation and extraction assays.

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THE DETERMINATION OF OTHER TITRATABLE ALKALOIDS IN THE MORPHINE OF OPIUM ASSAYS

Discussion

1. Progress in the opium assay through the years has in general resulted in an increase in the amount of morphine proved to be present in normal opium of various origins. We cannot, however, merely compare two different assay methods by their results, and conclude that the one yielding generally higher results is the better and more accurate. If the problem were as simple as that, it would only be necessary to increase the positive correction which is applied in several pharmacopoeial methods of assay, until there could be no question of its being high enough. We cannot even say that the precipitation method or the extraction method yielding the highest uncorrected results is the best of its class, until we have studied its possibilities of positive as well as negative errors.

2. While the ideal method will be free from substantial errors, either positive or negative, one less ideal in this respect may be shorter, faster, often useful, and generally satisfactory, provided that a true correction can be applied. A true correction can be ascertained if a balance-sheet can be drawn up experimentally for each type of opium, of positive and negative errors, and it can be applied in practice if the net correction is nearly enough the same for all types of opium, or if it is constant for particular types, and the type of opium is known at the time of the analysis. Such a balance-sheet begins to be possible with the use of the dichloracetic-acid separation introduced by the Secretariat.

3. Most methods of assay prior to the Secretariat's method explained in ST/SOA/SER.K/1, have included quite appreciable positive errors due to the presence of other titratable alkaloids contaminating the assay morphine. This applies both to precipitation methods and to extraction methods. The exceptions are perhaps, a few methods that have combined separation by extraction with precipitation of crystalline morphine from a relatively pure solution.

4. Examples of the last-mentioned methods are the method of Eder and Wäckerlin of 1937 (Quarterly Journal of Pharmacy and Pharmacology, 10, 690-730, 1937), the method of Eder and Wäckerlin of 1940 (Pharmaceutica Acta Helvetiae 15, 227, 1940), the method of Dowzard, Thomas, and Russo (Journal of the American

Pharmaceutical Association 26, 615-618, 1937), and the method of Baggesgaard-Rassmussen and Jespersen (Dansk Tidsskrift for Farmaci, 1937, 278-289).

5. Such methods doubtless are accurate, since the precipitated morphine is relatively pure and the correction for morphine remaining in the mother-liquor is relatively certain. However, they are complex and include most of the extraction methods. and yet give up the objective of determining the morphine without any correction. In short they are compromises, originating in recognition of the defects and undetermined errors of the usual precipitation methods, and inability to get the desired purity of the final morphine by extraction alone.

6. The simplest modification of the usual precipitation procedure by the use of an organic solvent was proposed by Annett and Singh in 1918, and consisted merely of separating the non-phenolic alkaloids from the line-water extract of the opium, by means of toluene, before precipitation. The morphine itself was not extracted. According to Annett and Singh, codeine causes the retention of additional morphine in the mother-liquor, and its removal thus diminishes negative as well as positive errors. The percentage of morphine by the British Pharmacopoeia process was increased by the toluene modification in assaying Indian opium. This article, "The effect of codeine in hindering the precipitation of morphine by ammonia from a solution of its lime compound" (Analyst 43, 205-213, 1918), has never had the attention it appears to deserve.

7. The straight precipitation methods, which are official in the Pharmacopoeias, include a positive error due to co-precipitation of other titratable alkaloids with the morphine, and often one due to titration of small amounts of lime compounds precipitated with the morphine. The latter is particularly discussed in "De Morphinebepaling in Opium" by M.A.G. Smeets (Thesis, Rijks-Universiteit, Utrecht, Netherlands).

8. These positive errors in precipitation methods are quite appreciable, even though they are greatly over-balanced by the chief negative error, the amount of morphine left in solution. Often a large positive correction is applied to compensate: but the correction, when one is used, is uniform, although the error certainly varies with the type of opium, if not even from sample to sample.

9. The determination of this principal error of precipitation methods has special difficulties, due to the accumulation of impurities with the relatively

small amount of morphine remaining unprecipitated. This problem will be studied later.

10. The other alkaloids in precipitated morphine have sometimes been determined, in the past, by extraction from alkali solution, usually with benzene. This was assumed to take out only non-phenolic alkaloids, and left the question unanswered, whether any of the minor phenolic alkaloids might be co-precipitated. As a matter of fact, such determinations of other alkaloids in the assay morphine are now found to be very nearly correct, at least for methods using lime-water extraction of the opium, because there is scarcely anything but codeine to be determined. However, the employment of chloroform extraction from dichloracetic acid solution rather than solvent extraction from alkali, for determining other alkaloids in precipitated morphine, gives assurance that contamination by the minor phenolic alkaloids is not being overlooked.

11. Determination of the impurities in the assay morphine have also been made in the past by determining the percentage of methoxyl present. The objection to this is that the methoxyl may be partly due to alkaloids that are not titratable, or even to non-alkaloidal substances.

12. Extraction methods were previously based on a separation by means of alkali solution and an organic solvent. It was generally taken for granted that the minor phenolic alkaloids accompanied the morphine to the final titration, but this possible error was commonly neglected by proponents of extraction methods, and over-emphasized - as an unknown quantity - by proponents of precipitation methods, or methods using precipitation after extraction.

13. Actually, as the Secretariat chemists have already shown, several of the minor phenolic alkaloids are separated from morphine by chloroform from alkali solution, though usually not (or at least not readily) by carbon tetrachloride or ether. However, an appreciable amount of phenolic alkaloid, about 0.3 per cent of the opium weight when calculated by the morphine factor, remains with the morphine even when chloroform is used. It consists chiefly of "alkaloid 3". When only a non-chloroformic immiscible solvent is used for this separation the positive error in the morphine determination will normally be greater.

Method

(For precipitation methods and for extraction methods other than the the Secretariat's dichloracetic-acid separation.)

14. After the titration of the assay morphine, if methyl alcohol was used to dissolve the morphine, evaporate the neutral solution just enough to drive it off. Then pour the solution into a separatory funnel, add a very little dilute H_2SO_4 in excess of neutrality, and shake out the indicator with two or three portions of CCl_4.

15. To the solution add 30 cc $CHCl_3$ and then $NaHCO_3$ to alkalinity to litmus, with a small excess; and shake. Transfer the $CHCl_3$ with shaking through the following solutions: in separatory #2 - 10 cc water plus 0.4 cc dichloracetic acid; #3 - 7.5 cc water plus 0.1 cc dichloracetic acid; #4 - 5 cc water (some dichloracetic acid is carried into this water by the $CHCl_3$); #5 - 10 cc water plus 10 drops concentrated NH_4OH; #6 - 10 cc wash water. In transferring the $CHCl_3$ from separatory #1 to #2, filter it through a pledget of cotton in a short-stemmed funnel. Finally filter the $CHCl_3$ from separatory #6 through a pledget of cotton into a weighed dish.

16. Shake out five times more with portions of $CHCl_3$ of 25 to 20 cc, then discard the original solution. Be sure that the solution in separatory #5 remains ammoniacal, and is not made acid by dichloracetic acid carried along.

17. If at any time it is considered necessary to check on the non-removal of morphine, take a tiny to small portion of the extract as it is transferred from separatory #5 to separatory #6. Evaporate the portion in a small white porcelain crucible, cool, and test with Frŏhde's reagent. No morphine reaction should be obtained. A different reaction from that of morphine is to be expected at first. When the Frŏhde test has become absent or negligible, test small portions also with Marquis' and Mecke's reagents (Codeine).

18. In the usual shake-out, test the sixth portion of $CHCl_3$ with Frŏhde's, Marquis', and Mecke's reagents, as just described. In shaking out the assay from an extraction process, if the tests indicate that some opium alkaloid is still being extracted, continue the shakeout from the dichloracetic-acid solutions with fresh portions of $CHCl_3$ (20 - 15 cc), until any other alkaloid is completely extracted, as long as morphine does not appear.

19. In the shake-out of the assay from a precipitation method, the tests on the sixth portion of $CHCl_3$ will probably show nothing but codeine still being extracted. In this case make the acid solutions (separatories 2, 3 and 4) alkaline with NaOH solution adding an excess. Two or three more portions of $CHCl_3$ will then remove the remaining codeine.

20. Finally, the $CHCl_3$ extracts in the weighed dish are evaporated. In the case of "other alkaloids" from a precipitation assay, some CCl_4 may then be added and evaporated off, whereupon, with a little continued heating on the steam bath, the residue will probably crystallize, as it is chiefly codeine. Cool the dish with the residue in a desiccator, and weigh. Then dissolve the residue in a little methanol, add methyl red indicator, and titrate with n/50 or n/100 H_2SO_4, with the addition of boiled distilled water. The residue of course is not morphine but may be calculated in terms of morphine, to express the result as an error of the morphine determination.

Method of ST/SOA/SER.K/1

21. The assay morphine of the method used by the Secretariat (ST/SOA/SER.K/1) has already undergone the dichloracetic-acid separation in the course of the assay. "Other alkaloids" can be determined in it in a similar way to the method just given, but substituting alkali solutions for the dichloracetic-acid solutions. Three alkali solutions are advisable, and the chloroform should be allowed to settle well after shaking and preferably be filtered from one separatory to the next, as traces of morphine are much more easily carried along by chloroform from alkali than is generally realized. The "other alkaloids" thus extractable yield a titration of only about 0.5 cc n/50 acid (equivalent to 0.07 per cent of the opium weight by the morphine calculation), when the dichloracetic-acid separation has been thoroughly carried out.

Some Results and Conclusions

22. The Secretariat chemists made a number of determinations of the titration of non-morphine alkaloid extractable by chloroform from dichloracetic-acid but not by chloroform from alkali. The results, beginning with the old League of Nations samples, (calculated as for morphine as percentage of the opium weight) were:

I.	Benares	0.33 per cent
III.	Iran	0.34, 0.35 per cent
IV.	Turkey	0.35 per cent
V.	Yugoslavia	0.30 per cent
Singapore sample (Far Eastern type of opium)		
		0.36, 0.32 per cent
	Japan	0.33 per cent

23. These determinations were discontinued as it seemed they could have no bearing on the origin problem, because the percentage was so nearly the same for different types of opium.

24. A determination of the other alkaloids in the assay morphine from a trial of the Buchbinder procedure showed 0.43 per cent of the opium weight as the positive error.

25. A few determinations were tried on the assay morphine of the Knaffl-Lenz method (ST/SOA/SER.K/3), but as the assays themselves were not altogether satisfactory, due to inexperience with the method, the results were somewhat inconclusive. In one case the "other alkaloids" found amounted to 0.93 per cent of the opium weight. This doubtless included some codeine, for, as pointed out in ST/SOA/SER.K/11 from the laboratories of S.E.M.P.A., a single extraction with benzene is not enough to eliminate even the non-phenolic alkaloids completely.

26. It is clear that any extraction method using merely the alkali separation, and which claims to have no negative error, needs a negative correction (to balance the positive error) of atleast 0.3 per cent of the opium weight.

27. This does not condemn such extraction methods in comparison to precipitation methods, for if the error is known, it is just as easy to make a negative correction as a positive one in the calculation; and perhaps considerably more accurate, having regard to the nature of the errors. However, such methods should be scrutinized very carefully to be sure that they really do not include any negative errors, before it is concluded that their results are necessarily too high.

28. The "other alkaloids" were determined by dichloracetic-acid separation on four assays by the method of the British Pharmacopoeia, two on an Indian opium and two on a Far Eastern opium with morphine contents of about 12 and 13.7 per cent.

The results in terms of the opium weight (calculated as for morphine) were 0.47 0.45, 0.55, and 0.42 per cent.

29. This material extracted from the B. P. assay morphine gave the analytical reactions of codeine and crystallized readily, as codeine. Scarcely more than traces of other alkaloids were present.

30. The results correspond closely to one stated by Rakshit (Analyst 56, 711-713, 1931). From 200 grammes of the assay morphine (B.P. 1914), he obtained 11.3 grammes of other alkaloids by extraction from alkali with benzene, i.e., 5.65 per cent by weight of the assay morphine. From 5 grammes of this material he obtained 3.6 grammes of crystalline codeine; there was no proof but that nearly all the rest may have been codeine, too. The total percentage in terms of anhydrous morphine calculated from titration would therefore be about 5.0 per cent of the morphine value.

31. The Secretariat results also correspond closely to the contamination found for U.S.P. assay by Rosin and Williams (Journal of the American Pharmaceutical Association 24, 1053-1062, 1931). By extraction with chloroform from alkali they obtained values (calculated as morphine as a percentage of the opium weight) of 0.45, 0.34, 0.53, 0.45, 0.39, 0.35 per cent; average 0.42 per cent.

32. The methoxyl determinations of Baggesgaard-Rasmussen, Jackerott, and Jespersen on the assay morphine of the International methods also correspond (Pharmaceutica Acta Helvetiae, Nr. 11, 1936). Calculated as codeine they represent 3.9 to 7.0 per cent of the morphine, representing an average, calculated as morphine error, of 4.85 per cent of the morphine value.

33. However, the contamination may often be greater. Smeets obtained higher methoxyl values than those cited. Recently the Saint Paul Laboratory of the United States Internal Revenue Bureau found U.S.P. assay morphine to contain 0.75 per cent of the opium weight (calculated as morphine), of non-morphine alkaloids, as determined by extraction from dichloracetic-acid. The Saint Paul Laboratory had obtained higher U.S.P. results on the particular sample than several of the other United States laboratories, which may possibly have a bearing on the high percentage of "other alkaloids".

34. It may be concluded that the "positive error" due to coprecipitation of other alkaloids is at least 0.4 to 0.5 per cent of the opium weight, and comes almost

entirely from codeine. In extraction methods using alkali separation it is nearly or quite as great, 0.3 to 0.4 per cent of the opium weight at a minimum, and due to phenolic alkaloids, especially "alkaloid 3". In the Secretariat's method, however, (ST/SOA/SER.K/1), the known "positive error" is reduced to less than 0.1 per cent of the opium weight. In the pharmacopoeial precipitation methods the "positive error" is overshadowed by the "negative error" of morphine remaining in solution, and this must be further studied.